

The role of the thymus in allogeneic haematopoietic stem cell transplantation

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Summary

Allogeneic haematopoietic stem cell transplantation (HSCT) is used to treat an increasing number of congenital and acquired disorders of the haematopoietic system. Even though cytoreductive conditioning regimens vary in intensity, all clinically used protocols invariably cause side effects that compromise transiently or long-term the response of the natural and the adaptive immune systems. However, in the context of the reconstruction of immunity, the generation of naïve T cells constitutes a slow process, and requires a functionally competent thymus. Unfortunately, regular thymic function is frequently suppressed by transplant-related toxicities. Most notably, graft-versus-host disease (GVHD) causes a state of posttransplantation immune deficiency. Here we discuss preclinical allogeneic HSCT models and clinical observations that have contributed to a detailed understanding of the cellular and molecular mechanisms responsible for the thymic dysfunction caused by acute GVHD. An in-depth knowledge of the mechanisms that control regular thymopoiesis and, conversely, affect thymus function is expected to provide the factual basis for the design of innovative therapies to recover T-cell numbers and function following allogeneic HSCT.

Key words: *graft-versus-host disease; haematopoietic stem cell transplantation; thymus; T-cell regeneration*

General principles of haematopoietic system replacement following HSCT

Allogeneic haematopoietic stem cell transplantation (HSCT) has become the standard therapy for an increasing number of congenital and acquired disorders of the haematopoietic system [1, 2]. In Europe, more than 10000 trans-

plants are now performed annually, the majority to treat leukaemias and bone marrow failure syndromes [3]. Patients eligible for allogeneic HSCT first receive preparative cytoreductive conditioning which is followed by the transfer of a donor haematopoietic cell graft. The stem cells contained in the graft allow complete replacement of the host's entire haematopoietic system with donor-type cells that gradually rebuild innate and adaptive immunity. Mature T cells contained in and transferred with the graft are of additional importance as they supply an instant, though transient source of functional immune competence. They can be directed against foreign antigens but also against residual cancer cells. The last-mentioned immune response has been referred to as graft-versus-cancer (or: graft-versus-leukaemia; GVL) effect and can be curative [4, 5].

Pre-transplant conditioning serves multiple objectives. Firstly, it is designed to reduce the bulk of malignant cells if HSCT is for cancer treatment. Secondly, it suppresses the host's immune system and thus prevents rejection of the donor cell graft. Finally, chemo-radiotherapy ablates host haematopoiesis, thus affording adequate space in the bone marrow for donor-derived haematopoietic stem cells (HSCs). Even though clinically used cytoreductive conditioning regimens vary in intensity, all protocols cause side effects that compromise immunity for a variable period after allogeneic HSCT. Consequently, patients are vulnerable to opportunistic infections [6, 7]. A delay in immune reconstitution can also be conducive to reactivation of latent infections, relapse of original disease or the development of secondary malignancies [6, 8]. Hence a favourable transplant outcome is dependent on swift reconstitution of the immune system, which ensures amendment

Abbreviations

Fgf7	Fibroblast growth factor-7 (Keratinocyte growth factor)
GVHD	Graft-versus-host disease
HSCT	Haematopoietic stem cell transplantation
K	Cytokeratin
RTE	Recent thymic emigrant
TEC	Thymic epithelial cell
TCR	T-cell receptor
TN, DP, SP	Triple-negative, double-positive and single-positive thymocytes
TREC	TCR rearrangement excision circle

of the transplant-related immune deficiency. Competent reconstruction of the immune system after HSCT depends on the ability of the donor HSC both to engraft and to generate anew the different cellular components of the haematopoietic system. In the absence of transplant-related complications, only a few weeks are required to replace the host's innate immune system [9]. For example, the time to exceed a threshold of 0.5×10^9 /litre peripheral blood granulocytes is usually reached between day 10 and 25 after transplantation. Such a value is taken as a measure of successful myeloid engraftment [10]. The blood count of monocytes, platelets, and natural killer cells typically normalises within the first 100 days, thus restoring much of the innate arm of the immune system in less than four months following HSCT. Infections that occur in the first month after HSCT chiefly result from a deficiency in myeloid lineage reconstitution [9]. Postengraftment infections that occur later are, however, indicative of a deficiency within the adaptive immune system [9].

Mature T cells are to a large extent destroyed by pre-conditioning, thus ablating the patient's competence to generate an adaptive immune response. An adequate posttransplant recovery of T lymphocytes is associated with enhanced survival and hence can be used as a predictive factor for transplant outcome [11–16]. Renewal of the lymphoid compartment is a more complex and decidedly slower process when compared to the replacement of the myeloid system. Pretransplant B-cell frequencies are usually reached in HSCT recipients only after several months. Successful reconstruction of the T-cell compartment requires, however, one to two years and depends on distinct but mutually non-exclusive pathways (fig. 1). The profound lymphopenia caused by conditioning favours first and foremost thymus-independent rapid expansion both of the few surviving host T cells and of the mature donor T cells that have been transferred with the graft. Proliferation in response to homeostatic signals and the oligoclonal expansion in response to alloantigens (see below) or nominal antigens explains the numerical recovery of T cells [17–19]. They are also of functional relevance since donor-derived memory T cells generated in response to specific pathogens and then transferred with the HSC graft can transiently protect against viral infections (e.g., CMV, EBV)

[9]. This protective potential may be a consequence of the ability of transferred memory T cells to migrate from the circulation to selected peripheral sites, such as the intestines, skin and lungs [20]. These and other features that adaptively transmit immunological competence early after HSCT have been exploited for immunotherapy to supplement allogeneic HSCT [21]. The resultant antigen-mediated T-cell expansion is, however, transitory as the adoptively transferred cells undergo apoptosis as a result of their extensive replication [18, 22, 23].

The second pathway used to replete the periphery with T cells requires a functionally competent thymus able to recapitulate T-cell ontogeny. Following lymphodepletion and HSCT, lymphoid progenitors come from the bone marrow to the thymus where they commit to T-cell lineage development and differentiate into phenotypically and functionally mature T cells [24]. Upon their export to the periphery, these naïve cells establish a donor-derived T-cell compartment characterised by a broad T-cell antigen receptor (TCR) repertoire which responds to foreign antigens but is tolerant to host tissues (fig. 1).

Thymic function in the context of HSCT has recently received considerable attention because of the growing appreciation that conditioning, graft-versus-host disease (GVHD) and other transplant associated toxicities affect T-cell reconstitution. A detailed understanding of the molecular and cellular mechanisms that control regular thymopoiesis and, conversely, affect thymus function are expected to provide the basis for the design of innovative therapies that boost thymic function following allogeneic HSCT.

Normal thymus function: T-cell development and export

The thymus is the primary lymphoid organ responsible for the lifelong generation of T lymphocytes [25, 26]. Its structural organisation is highly conserved between different vertebrate species and reveals morphologically distinct subcapsular, cortical and medullary regions. As the thymus does not contain self-renewing haema-topoietic precursor cells, there is a need for progenitor cells to be continuously recruited from the blood. T-cell precursors enter the

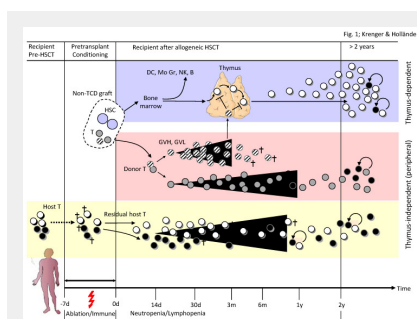


Figure 1

Schematic representation of T-cell regeneration after non-T-cell depleted (TCD) allogeneic HSCT. Thymus-dependent and thymus-independent pathways contribute to reconstruction of the host T-cell compartment following lymphodepletion and allogeneic HSCT. Initial numerical recovery is imparted by conditioning-resistant naïve (○) and memory (●) host T cells and by mature non-alloreactive T cells (●) contained in the donor haematopoietic stem cell (HSC) graft. T cells of either origin expand in the periphery and may provide early posttransplant immunity. However, replicative senescence limits their expansion. Surviving T cells from either source will contribute to the memory T cell pool. The donor graft also contains mature alloreactive T cells (●). These cells expand rapidly in response to host tissue alloantigens and account for graft-versus-host (GVH) and graft-versus-leukaemia (GVL) responses. Parallel to the

peripheral oligoclonal expansion of mature T cells, engrafted donor HSC give rise to precursors that enter the thymus where they de novo generate T cells. This process allows the export of naïve T cells (○) with appropriate receptor specificities. In the presence of a functional thymus and in the absence of significant transplant related toxicities, a sizeable pool of naïve T cells is generated within 1–2 years after allogeneic HSCT. As the process of peripheral expansion gradually converts naïve T cells to memory T cells, a continued thymic export of newly generated T cells is required to replenish peripheral lymphoid tissues with naïve T cells. During acute GVHD thymic export is, however, limited because alloreactive T cells cause injury to the thymus.

thymus at the cortico-medullary junction as cells with a CD3⁺CD4⁺CD8⁺ (triple negative, TN) phenotype (fig. 2). Following their entry, they gradually change their phenotype but initially remain TN cells and eventually concomitantly express the cell surface markers CD4 and CD8, a phenotype designed double-positive (DP) [27]. During differentiation, thymocytes are subjected to a strong expansion phase [28]. The assembly of a complete TCR complex (composed for the vast majority of T cells of an α and a β chain) renders DP cells subject to thymic selection. This process allows the formation of mature single CD4 and CD8 positive (SP) thymocytes expressing TCRs that are collectively responsive to a seemingly unlimited array of foreign ("non-self") antigens. At the same time, the process of thymic selection also assures that the emerging T cells are tolerant to the host's own tissues ("self"). After residence in the thymic medulla to complete post-selection maturation, naïve SP T cells emigrate as recent thymic emigrants (RTE) to peripheral lymphoid tissues. For the transitions through the separate developmental stages to be successful, thymocytes rely on different signals that are provided by a regularly structured and normally composed thymic stromal network [29].

Function of the thymic stroma

All non-lymphoid cells of the thymus are commonly referred to as stroma and collectively form a microenvironment that is critical for the essential capacity to attract T-cell precursors, to control their further maturation and enforce their selection. Thymic epithelial cells (TEC) constitute the major cellular component of the stroma [30, 31], which in addition entails reticular fibroblasts, dendritic cells, macrophages and other cell types. TECs lack the typical cell polarity of epithelia, possibly because they are not placed on a basal membrane but arranged in a three-dimensional network where single cells contact each other via dendrite-like cell processes. Thymic epithelia are phenotypically and functionally heterogeneous, with different subpopulations not only present in the anatomically sep-

arate compartments of cortex and medulla but also existent within these domains. While a detailed TEC analysis has not yet been reported for the human thymus, lineage- and development-specific cell markers and cell kinetic data have been described for several experimental animal models and make it possible to distinguish separate TEC subpopulations [32]. For example, the expression of cyto-keratins 5 (K5) and K18 in conjunction with the detection of the UEA-1 binding lectin and other cell surface markers renders possible differentiation in the mouse between cortical (K5⁺K18⁺UEA-1⁺) and medullary TEC (K5⁺K18⁺UEA-1⁺).

The differentiation and proliferation of embryonic and postnatal TECs depend on distinct signals including the availability of the epithelial cell mitogen Fgf7 (fibroblast growth factor-7; a.k.a. keratinocyte growth factor) [33, 34]. TECs expand rapidly throughout mouse foetal development and during the first four weeks of life. Once a steady state has been reached, TECs are continuously replaced, resulting in their complete turn-over within 10–14 days [32, 35]. The proportion of TEC compared to thymocytes progressively decreases with age from a value of one TEC to approximately 440 thymocytes at four weeks of life to a ratio of one TEC to 320 thymocytes after puberty [32]. These kinetics are comparable to those observed in man, where thymus size and hence TEC cellularity at first expand but then decrease with the second year of life [36, 37].

TECs play an important role in attracting blood-borne lymphoid precursors, enforcing a commitment of these cells to a T-cell lineage fate and controlling subsequent thymocyte development. In this context it is also the interactions between immature thymocytes and TECs that are critical for the selection of a useful TEC repertoire among the evolving T cells, since cortical TEC present an array of self-peptides/MHC complexes [38, 39]. DP thymocytes with a TCR that recognises any of these complexes with a sufficiently high affinity will survive a process designated positive thymic selection. In contrast, DP thymocytes will undergo programmed cell death should their TCR fail to recognise peptide/MHC complexes with sufficient affin-

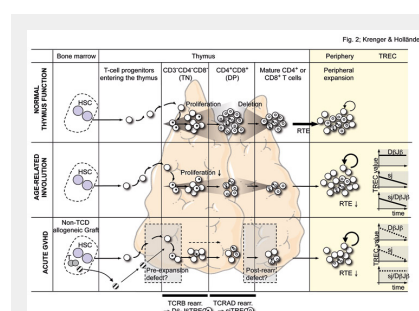


Figure 2

Thymic export and its detection via TREC analysis under steady-state conditions, in aged individuals and during acute GVHD. The top panel displays a scheme for thymus function in young individuals. The HSC-derived lymphoid progenitors (○) enter the thymic microenvironment where they are committed to the T-cell lineage. Mouse cells at early stages of this development are phenotypically characterised by the absence of CD4, CD8 and T-cell receptor cell surface expression (designated triple negative, TN thymocytes). At this developmental stage, these cells not only undergo TCRB gene locus rearrangement leading to Dβ-JβTREC formation (filled circles within TN cells) but also proliferate substantially. In the subsequent DP stage, rearrangement of the TCRA/D generates sjTREC (open circles). The sj/DβJβ ratio can be used as a marker for intrathymic proliferation and for thymic export. As the peripheral T-cell proliferation does not change the sj/DβJβ ratio, its measurement among peripheral blood cells provides a molecular tool to quantify the number of recent thymic emigrants (RTE). The middle panel depicts T-cell development during age-related thymic involution. Here, the altered thymic microenvironment leads to a decrease in TN proliferation that is detected by a gradual decline in the sj/DβJβ ratio (as only the sjTREC but not the Dβ-JβTREC frequency decreases over time). Diminished thymic export into the periphery is compensated by enhanced T-cell proliferation (arrow circle). However, homeostatic proliferation converts cells with a naïve phenotype into cells typical of the memory T-cell pool. The lower panel shows thymic dysfunction during acute GVHD. Donor-derived alloreactive T cells (●) invade the thymic micro-environment and cause structural and functional injury to it. The consequent impairment in T-cell development is marked by a decrease in both the Dβ-JβTREC and sjTREC frequencies, leading to a stable sj/DβJβ ratio over time. Hence acute GVHD causes changes that either occur at the T-cell progenitor stage prior to Dβ-JβTREC formation or take place after sjTREC generation (e.g., apoptosis of postrearrangement DP thymocytes).

ity. This alternative fate has been termed “death by neglect” because the survival of immature thymocytes is critically dependent on the provision of TCR-mediated signals if it is to escape the otherwise unavoidable fate of apoptosis. Positively selected thymocytes that have migrated from the cortex to the medulla are subject to yet another TCR selection step. Designated negative thymic selection, this process depletes SP thymocytes that express a high affinity TCR for self-antigens and hence have the potential to elicit autoimmunity [40]. Negative thymic selection is effected by medullary TEC and dendritic cells which are enriched at the cortical-medullary junction (i.e., the gateway to the medulla), and dispersed throughout the medulla. The capacity of medullary TEC to express a broad array of self-antigens typically expressed by other (i.e., peripheral) tissues constitutes an important requirement for the negative selection of thymocytes with an autoreactive TCR specificity. This phenomenon has been termed promiscuous gene expression and allows medullary TECs to present a “molecular mirror of peripheral self”. Promiscuous gene expression is therefore critical in rendering SP thymocytes tolerant, either by the aforementioned deletion of reactive T cells or via generation of regulatory T cells (designated T_{reg}) which actively suppress a response to self-antigens [41, 42]. Natural (i.e., thymus-derived) T_{reg} are characterised by their signature $CD4^+CD25^+$ phenotype and the expression of the transcription factor Foxp3 [43, 44]. A deficiency in negative thymic selection and/or a breakdown in the generation of natural T_{reg} contribute to loss of immunological tolerance and consequently lead to overt autoimmunity [45, 46].

Thymic export and its measurement under steady-state conditions

The number of total peripheral T cells is kept constant in the absence of external stimuli by a mechanism known as homeostatic proliferation [47]. At the same time, the thymus continues to export naïve T cell to peripheral lymphoid tissues [48]. Because cell surface markers accurately identifying RTE in humans or in mice are unfortunately unavailable, it has been difficult to determine truthfully the extent of thymic export under both physiological or pathological conditions. This limitation has, however, been recently overcome with the development of a molecular method to determine thymopoietic activity. During development, two families of circular DNA products are generated while immature thymocytes undergo DNA rearrangement to build either the TCR β -chain from the TCRB locus or the TCR α -chain from the TRAD locus [49–53]. These rearrangements form TCR rearrangement excision circles (TREC) which are distinct in relation to their nucleotide sequence and to the cells’ developmental stage at which they are generated: The TCRB locus is recombined in TN thymocytes and forms D β -J β TREC whereas the TRAD locus is rearranged in DP thymocytes and generates sjTREC [54]. Since the TCRB recombination occurs prior to TN expansion and because episomal DNA circles cannot replicate with cell division, D β -J β TREC are progressively diluted among TN thymocytes as these cells proliferate prior to adopting a DP phenotype. The sj/D β -J β TREC ratio (sj/D β J β) thus provides a quant-

itative marker for the proliferation of thymocytes that have rearranged their TCRB locus [52]. Because the magnitude of TN expansion is the key determinant for thymic cellularity which in turn establishes the extent of thymic output [55], it is the sj/D β J β ratio measured among peripheral T cells that serves as a suitable marker to quantify thymic output [56, 57] (fig. 2). Importantly, this ratio is independent of peripheral T-cell expansion and thus avoids difficulties previously encountered when attempting to assess thymic output by the determination of only sjTREC [50].

Thymus-dependent T-cell regeneration after lymphoablation and HSCT

Deficits in both number and function of peripheral T cells following HSCT are thought to be in large part a consequence of inadequate thymic function. Hence, for the design of novel therapeutic strategies to improve T-cell reconstitution, a detailed understanding is necessary of how thymic mechanisms determine a swift and comprehensive reconstitution of the T-cell compartment and how exogenous factors adversely influence thymopoiesis [6, 7].

Thymus-dependent T-cell regeneration in human allogeneic HSCT: an issue of numbers ...

The correction of T-cell lymphopenia following HSCT depends on two parallel pathways, the extrathymic expansion of mature T cells and the thymus-dependent *de novo* generation of T cells (see fig. 1 and references [6, 21, 58]). These two mechanisms influence each other but peripheral T-cell expansion dominates when the thymus is not functioning properly [59–63]. An intact thymus-dependent generation of T cells is characterised by the emergence of naïve ($CD45RA^+CD62L^+$) TREC-positive T cells [6, 8, 14, 49, 61, 63–69]. Even under most favourable conditions, several weeks are required to produce mature naïve T cells from bone marrow-resident precursors [70, 71]. It is thus not surprising that repletion of the T-cell compartment with new T cells is a time-consuming process. A minimum of two to three months is needed after transplantation to accumulate a meaningful number of naïve T cells that can be detected in the peripheral blood of transplanted individuals [72]. Intriguingly, this time is comparable to the period required for thymus-resident precursors to become peripheral mature T cells during human foetal development. For 1–2 years after HSCT the number of mature T cells continues to rise (fig. 1). Peripheral blood $CD8^+$ T cells may reach normal levels as early as 3–6 months whereas a substantially longer period is necessary for sufficient $CD4^+$ T cells to emerge [73, 74]. The thymus-dependent pathway of T-cell renewal is much more effective in younger HSCT recipients [36]. This observation has been linked to the fact that the thymus undergoes an age-dependent process of involution that is marked by structural remodeling [37]. The thymus of older individuals displays in parallel a lower TN proliferation (as detected by a gradual decline in the sj/D β J β ratio [52]) and a decrease in T-cell export (fig. 2)). The kinetics and the plasticity to regain thymopoietic function after HSCT are also dictated by the recipient’s age [75]. These limitations are of clinical relevance as they are likely to be responsible for the increased incidence of

immunodeficiency and autoimmunity observed in elderly HSCT recipients [76].

... and of quality

Protection against infectious pathogens and potentially anti-tumour responses correlate with the presence of antigen-specific immunity, not the immunophenotypic presence of T cells. Hence it is a key goal of transplantation immunology to ensure the development of a diverse post-transplantation TCR repertoire, the quality of which is known to dictate clinical course and outcome of HSCT [11, 19, 77–79]. The post-transplant quality of the T-cell compartment is best assessed by the determination of TCR diversity and T-cell function. The repertoire of the variable gene segments used for the β -chain of the TCR is either measured by flow cytometry or assessed by a PCR-based analysis (known as spectratyping of the complementarity determining region 3, CDR3) [68, 77, 80–82]. To determine their function, T cells are stimulated *ex vivo* and their capacity to secrete cytokines is quantified (Elispot). Alternatively, the frequency of antigen-specific T cells can be measured by fluorescence-labeled tetrameric antigen/MHC complexes.

The genetic origin of the stem cell graft substantially influences the quality of T-cell reconstitution: recipients of HLA-identical, related HSC display only a mild perturbation of their TCR repertoire, whereas a skewed TCR repertoire (at least for the first few years after transplantation) is noted in recipients of HSC isolated either from unrelated or HLA-mismatched, related donors [73, 81, 83–88]. The selection of the TCR repertoire during thymocyte maturation depends on is the expression of peptide/MHC complexes by haematopoietic and epithelial stromal cells. Under physiological conditions these cells express, independently of their origin, an identical MHC haplotype which ensures that positive and negative selections are instructed by the same MHC molecules. Moreover, foreign antigens presented for immune recognition by antigen presenting cells in peripheral tissues will be complexed to those MHC molecules that have already been used for TCR selection. This situation changes in allogeneic HSCT where the MHC haplotype of the donor graft is partly dissimilar from the recipient's. The radioresistant cortical TECs needed for positive selection remain of host origin even after transplantation, whereas the cells required for negative thymic selection express two distinct MHC haplotypes. The autochthonous radioresistant medullary TECs remain of recipient origin while the radiosensitive dendritic cells and macrophages are derived from the donor graft and hence express the MHC haplotype of the donor. This variance in MHC restriction is thought to influence thymic T-cell repertoire selection and, as a result, impact on the quality of T-cell reconstitution in the HLA-nonidentical recipients described above.

Transplant-related toxicities and their impact on thymic function

Treatment-related adverse events are of clinical importance as they determine transplant-related mortality [89]. Among the most prominent transplant-related toxicities are (i) development of acute and chronic GVHD and (ii) injury

caused by cytoreductive conditioning. Here we will discuss how these toxicities influence the thymus-dependent pathway of T-cell reconstitution.

Human thymic function in the context of acute GVHD

GVHD remains the major complication of allogeneic HSCT and presents as an acute and/or chronic pathology. Differences in the cellular and molecular pathomechanisms account for the separate clinical features of acute and chronic GVHD respectively [90, 91]. Acute GVHD is induced by alloreactive mature T cells which are present in the HSC graft either inadvertently or by design. Upon activation by host alloantigens, these donor-derived cells mediate an inflammatory immune response causing cell injury in a limited number of target tissues via antigen-specific (anti-host cytotoxic T lymphocytes) and non-specific (cytokines) effector mechanisms. While clinical attention has initially centred on the involvement of skin, liver and gastrointestinal tract, recent interest has also focused on the host's lymphohaematopoietic system as an important target of acute GVHD [92].

Acute GVHD in allogeneic HSCT recipients constitutes a major risk factor for opportunistic infections [65, 86, 93–97], not least because GVHD severity is inversely correlated to the thymic capacity to generate naïve T cells. Indeed, clinical observations more than thirty years ago had identified the thymus as a target of acute GVHD [98–102]. As a consequence of thymic GVHD, the number and composition of the different TEC subpopulations are altered, the cortico-medullary demarcation is lost, and the Hassall's bodies are eliminated [98–102]. These alterations of the thymic stroma correlate with a partial depletion of thymocytes, diminished thymic export and a distorted TCR repertoire [56, 65]. In patients with acute GVHD, impaired thymic function is characterised by a comparable decrease in D β -J β TREC and sjTREC frequencies, which result in a normal sj/D β J β ratio in peripheral blood T cells [56, 57] (fig. 2). These changes suggest that acute GVHD affects thymopoiesis either at a stage prior to TN proliferation or at a time when both TCR chain rearrangements have already occurred (i.e., after the late DP cell stage). With the successful resolution of acute GVHD in young patients, a recovery in both types of TRECs and thus in thymic export can be observed [56, 57]. Importantly, TREC measurements distinguish thymic GVHD from age-related thymic involution as the latter is marked by an increased sj/D β J β ratio secondary to diminished TN proliferation [52].

Pathomechanisms of thymic insufficiency: Insights from acute GVHD models

Preclinical allogeneic HSCT models have been in many ways informative in delineating the cellular and molecular mechanisms responsible for the thymic dysfunction caused by acute GVHD. The morphological features of experimentally induced acute thymic GVHD correspond to those detailed above for humans [58, 103]. Moreover, the widespread alterations in the cellular composition and architectural organisation of the thymic stroma have been causally linked to deficient T-cell development and selection [104–106]. Consistent with clinical data [56, 57], the defects to the thymus stroma set off by experimental GVHD

affect two different stages during mouse T-cell development, pre-TN proliferation and the survival of selected DP thymocytes [104, 105] (fig. 2). These mechanisms in combination lead to thymus hypoplasia and diminished thymic export [106].

The alloantigen-specific recognition of host TECs by donor T cells provides the principal injury that impairs thymocyte maturation and selection. In fact, mature donor T cells infiltrating the thymus have long been recognised as a typical feature of acute GVHD [99] and alloreactive donor T cells from the graft are able to access the host's thymus even in the absence of conditioning [104, 107]. The extent by which thymopoiesis is disturbed correlates with the number of donor T cells that have accumulated *in situ*. In contrast, cytokines or glucocorticoids released in the non-specific inflammatory effector arm of acute GVHD do not appear to have a role in the pathophysiology of thymic GVHD [104, 105].

The unique biology of TECs as antigen-presenting cells authorises them to prime allogeneic T cells even in the absence of professional haematopoietic antigen-presenting cells [97, 107] (fig. 3). Following their activation by allogeneic TEC, thymus-resident donor T cells secrete IFN- β which in turn initiates – via signal transducer and activator of transcription (STAT)-1 – the programmed cell death of both cortical and medullary TECs. Consequently, mostly immature thymocytes fail to receive the required epithelial survival and differentiation signals (e.g., interleukin-7), their entry into the cell cycle is blocked and they consequently begin to die. Thymic hypoplasia and diminished export of naïve T cells are the consequence of these changes and result in peripheral T-cell lymphopenia. However, the structural and functional alterations to the thymus are not easily assessed by conventional diagnostic tools, which renders the monitoring of thymic GVHD difficult.

The fact that TECs serve both as initiators and targets of acute thymic GVHD has implications for the design of novel strategies to prevent allorecognition by mature donor T cells. Firstly, it is unlikely that a vigorous depletion of host-derived haematopoietic antigen-presenting cells (such as dendritic cells) will prevent allogeneic T-cell priming and avert the ensuing TEC damage. Future therapeutic efforts may therefore wish to focus on strategies aimed at the prevention and/or repair of the epithelial injury (see below). If successful, such endeavours are likely to shed light on the current uncertainty as to whether increased susceptibility to infections and incomplete reconstitution of the adaptive immune system in recipients of allogeneic HSCT are caused by subclinical GVHD exclusively restricted to the thymus.

Enhancing T-cell immune regeneration via TEC protection: proof of concept

Strategies aimed at enhancing thymic function hold out promise of ameliorating posttransplant T-cell immunodeficiency [108–112]. Both prophylactic and therapeutic approaches may be effective in enhancing (residual) thymic function or, alternatively, generating new thymic stromal elements. Several tactics have so far been proposed for and tested in preclinical models. However, few of these have so

far been successfully translated into clinical practice. Here we will discuss interventions directed at preventing TEC injury or enhancing TEC function after allogeneic HSCT.

Fgf7 (palifermin® [33, 34]) is currently an approved drug for the prophylaxis of oral mucositis in conditioned transplant recipients [113, 114]. This epithelial growth factor belongs to the large structurally related family of the Fgfs [115]. To exert its biological activity, Fgf7 binds to and activates the IIIb variant of the FgfR2 receptor (FgfR2IIIb), which in the thymus is exclusively expressed on TECs [116–120]. In response to systemic Fgf7 treatment, the post-natal thymic microenvironment of normal mice undergoes within days specific changes, including a robust expansion of both mature and immature TECs without compromising their architectural organisation [119]. The resultant enlargement of the TEC scaffold accommodates a higher lymphoid cellularity while keeping a normal thymocyte/TEC ratio. Hence, exposure of mice of any age to exogenous Fgf7 causes an increase in thymopoietic activity and reconstitution of the peripheral T-cell compartment in syngeneic or allogeneic transplant recipients preconditioned with chemo- or radiotherapy [118, 121]. When used in preclinical models of acute GVHD in the absence of preconditioning, the systemic administration of Fgf7 preserves TEC architecture, cellularity and function [117]. As a result, normal thymic T-cell development is maintained regardless of the *in situ* presence of alloreactive donor T cells and ongoing GVHD in other typical target organs.

The potential for beneficial use of palifermin in clinical HSCT is further underscored in a nonhuman primate model where Fgf7 given prior to myeloablation and autologous HSCT increases thymopoiesis and peripheral immune system recovery [110]. These results indicate that the beneficial immune system effects of palifermin are not restricted to small animal models. Conclusive data are, however, still lacking in human recipients of an allogeneic HSCT. An initial phase I/II study could not demonstrate a advantageous effect on haematopoietic reconstitution [109] although thymic function was not specifically investigated. Fgf7 fails to protect completely medullary TEC or to restore CD8⁺ T-cell numbers and function in allogeneically transplanted mice [122]. Studies have therefore been initiated in animals with a view to assessing whether T-cell recovery may be enhanced by combined use of Fgf7 with other interventions such as chemical androgen blockade [122, 123]. The latter is used because senescence-driven thymic involution is linked to changes in sex steroid hormone production [124–126]. Androgen receptors are indeed expressed on TECs and their binding to sex steroid ligands inhibits thymopoiesis [127]. Another study [122, 123] focused on the combination of Fgf7 with PFT- β , a blocker of the p53 tumour suppressor protein, which has been independently approved for clinical trials to ameliorate the general side effects of chemotherapy. Data from these experimental studies raise reasonable hope that a combination therapy which includes Fgf7 may be used to enhance T-cell reconstitution in lymphopenic recipients of allogeneic HSCT. The molecular mechanisms by which Fgf7 influences TEC function are, however, not yet fully established but are the current focus of intense pre-clinical investigations.

The thymus does not sense peripheral T lymphopenia and is hence unable to gauge its T-cell export accordingly. Thus, establishing means of increasing thymic T-cell export in lymphopenic individuals is clinically desirable because any increase/enhancement in thymic output should positively affect the process of replenishing the peripheral T-cell pool. Enhanced thymic function should also secure a diverse T-cell repertoire since it will concurrently offset the skewed TCR repertoire of T cells that have been expanded in a thymus-independent fashion.

Conclusion

The indications for HSCT have steadily increased in recent years. Correspondingly, the number of patients affected by transplant-related toxicities including GVHD is expected to rise. Experimental evidence has unequivocally demonstrated that the thymus constitutes a primary target for GVHD. Indeed, in mice alloreactive donor T cells mediate severe changes in the composition, architectural organisation and thymopoietic function of the thymic epithelial compartment. This impairment to the stroma negatively affects the maturation, TCR repertoire selection and export of T cells. Although yet to be tested in humans, therapeutic strategies that enhance TEC numbers and function may help to promote a diverse T-cell repertoire and hence the generation of a functionally competent adaptive immune system, thus improving the outcome of clinical allogeneic HSCT.

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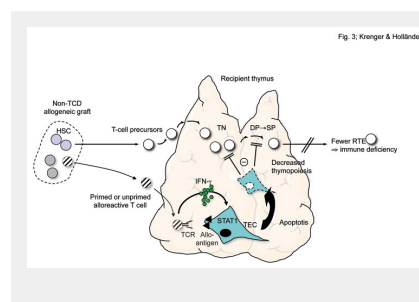


Figure 3

How killing of thymic epithelial cells (TECs) by alloreactive T lymphocytes can lead to post-transplantation immune deficiency. Alloreactive primed or unprimed naïve T lymphocytes enter the recipient thymus after allogeneic HSCT. As a result of T-cell receptor (TCR)-mediated recognition of alloantigens presented by TECs, donor T cells secrete interferon (IFN)- γ . In response, TEC activate the STAT1 transcription factor and initiate an apoptotic programme leading to TEC death. The loss of TECs results in a structurally and functionally defective microenvironment unable to support normal thymopoiesis. The resultant decrease in thymic export ultimately correlates with post-transplant immune deficiency.

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